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IN VITRO MULTIPLE SHOOT INDUCTION FROM EMBRYONIC AXES OF ANNUAL HERBACEOUS LEGUME FABA BEAN (*Vicia faba* L.)

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Abstract: The faba bean (*Vicia faba* L.) is cultivated in the Mediterranean and Aegean regions only. It is a rich source of protein and an important source of food and feed for human and animal consumption. Faba beans have a narrow genetic base and their improvement through hybridization is not feasible because of high pollen self-incompatibility. This study was carried out using the embryonic axis of cv. Filiz99 and Eresen87 regenerated on MS medium containing 11 different combinations of BAP and NAA. The results showed 100% shoot regeneration frequency with maximum number of 3.3 and 3.5 shoots per explant on cv. Filiz99 and Eresen87 respectively. Regenerating shoots were rooted on 1 mg L–1 IAA. The in vitro regenerated shoots were continuously cultured for 3 weeks to acclimatize them. This approach could improve broad bean seed germination and subsequently regeneration. The results could also facilitate genetic transformation studies.

Keywords: Acclimatisation, BAP, NAA, Zygotic embryos

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1. Introduction

Faba bean (*V. faba* L.) is a herbaceous multipurpose herbaceous legume plant grown in many parts of the world (Duke 1981). It is economically important for food, fodder, and stabilization of soil particles in high wind erosion areas. It is widespread in North Africa, Europe, China, Central Asia, the USA, Canada, South America, and Australia. Worldwide Faba bean production for food and feed was 4.5 million t in 2012 (FAO, 2017). The 5 top (75% of world production) producing countries are the United Kingdom, France, Australia, Ethiopia, and China. It is an introduced crop in Turkey with plantations in the Mediterranean and Aegean regions of Turkey (Rahate et al., 2020). Almost no work on in vitro micropropagation of faba bean has been carried out in Turkey.

Tissue culture is generally used for cloning of superior genotypes and in breeding programs of herbaceous perennials (Karnoski, 1981; Boulay, 1987). There is a need to make attempts to regenerate these important herbaceous legumes through tissue culture. Cv. Filiz 99 and cv. Eresen 87 are two important varieties that are grown in Turkey for commercial food and feed production. Like other faba beans (Rowlands 1964), these are less genetically diversified cultivars and face problems of partial self-incompatibility that more often results in the collapse of their fertile ovules. This has also led to slow progress in broad bean breeding and varietal improvement activities in Turkey (Bond, 1987; Selva et al., 1989; Bahgat et al., 2009). Although a few reports on tissue culture of faba bean are available, there has been no report of tissue culture from Turkey and it is in fact an important issue. Faba beans are highly susceptible to biotic and abiotic stresses and have instability in yield that makes this crop less attractive (Bahgat et al., 2009). More often the problem of partial self-incompatibility results in the collapse of fertile ovules in broad bean resulting in genetically less diversified plants (Rowlands, 1964; Stoddard and Bond, 1987). This has also led to slow progress in broad bean breeding and varietal improvement activities (Bond, 1987; Selva et al., 1989; Bahgat et al., 2009).

Broad bean appears to be recalcitrant towards in vitro regeneration and tissue culture (Khalafalla and Hattori, 2000; Anwar, 2007; Anwar et al., 2010), due to the presence of inhibitors, high oxidative stress, and release of phenolic compounds (Böttinger et al., 2001). Due to instability among genotypes, most of the reported protocols are variety/cultıvar specific. Although there have been many reported studies of successful regeneration in faba bean (Shri and Davis, 1992; Rizvi and Singh, 2000; Polowick et al., 2004), most of the protocols are not repeatable. There is a need to overcome these problems to enhance shoot regeneration. Successful establishment of a reliable in vitro regeneration protocol could help in accelerated breeding. Establishing faster regeneration systems can be used in

combination with traditional broad bean breeding techniques (Kuchuk, 2001).

The present communication aims to identify efficient, repeatable protocol for in vitro shoot multiplication from cv. Filiz 99 and cv. Eresen 87 of *V. Faba* in development of accelerated regeneration technology.

2. Material and Methods

2.1. Seed Source and Surface Sterilization

The widely cultivated in Turkey seeds of broad bean cv. Eresen 87 and Filiz 99 were obtained from the Aegean Field Crops Research Institute, Izmir, Turkey.

Cv. Eresen 87 has an average seed weight of 1.84-190 g, seed width of 1.74 -1.81 cm, seed length of 2.44 to 2.52 cm, and seed thickness of 0.61 to 0.65 cm. It has a thousand-grain weight of around 1350-1600 g with variable seed yield between 2000-5000 kg/ha depending on sowing time and environmental conditions. Cv. Eresen-87 is consumed both as a green vegetable and dry grains. The grains of the Eresen-87 variety used in the study are flat, light brown, black, and the weight of 100 is 135-160 g. Plant type is vertical, plant length is 90-107 cm, with 12-19 cm long beans. It is a medium early cultivar and tolerant to anthracnose and rust (Yaman, 1996, Pekşen and Artik, 2006; Alan and Geren, 2006).

The grain of cv Filiz-99 is flat with yellowish-brown color and black hilum. It has 100-grain seed weight of 115-125 g. It grows vertically with a plant height of 85-102 cm and bean length of 12-14 cm. cv. Filiz 99 has an average seed weight of 1.42 -1.51 g, seed width of 1.49 -1.58 cm, seed length of 2.00 to 2.13 cm, and seed thickness of 0.52 to 0.58 cm. It is harvested earlier and is moderately resistant to anthracnose and susceptible to chocolate dust disease (Pekşen and Artik, 2006; Alan and Geren, 2006).

Healthy and clean seeds were selected and subjected to surface sterilization using 60% commercial bleach (Ace®Istanbul, Turkey, containing 5% NaOCl) for 20 min followed by 3×5 min rinsing with sterile distilled water. These embryos were taken out from these seeds with soaking them in sterile water for 24 h at 24°C.

These were cultured on MS medium (Murashige and Skoog 1962) containing 0.00 (control), and 0.25 mg L^{-1} BAP+ 0.00, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25 mg L^{-1} NAA (11 different combinations).

All cultures were autoclaved for 20 min at 121˚C, using 105 kPA autoclave pressure. The pH was adjusted to 5.6-5.8 with1M NaOH or 1M HCl. All cultures were grown at 25±2˚C with a 16 h light photoperiod. Light was maintained at intensity of 45 µmol $m^{-2}s^{-1}$ by cool white fluorescent lamps.

2.2. Evaluation of seeds for SOD, CAT and POD Antioxidant Enzymes' Activity

These tests were performed using 500 mg of leaf tissues obtained from seedlings grown from non-water-soaked and water-soaked seeds. The leaf tissue were homogenized to prepare a crude enzyme extract in extraction buffer having 100 mM potassium phosphate buffer, pH 7.0 containing and 1% polyvinylpyrrolidone and 0.5% Triton X-100 using mortar and pestle chilled in liquid Nitrogen. It was followed by centrifuging this homogenate at 15000 rpm for 20 min at 4 °C. Thereafter, the resulting supernatant was used for each of the enzymatic assays as given with expression in milligrams of protein per minute (Chance and Maehly, 1955).

SOD (EC 1.15.1.1) activity was measured following Giannopolitis and Ries (1977) based on the inhibition of nitro blue tetrazolium (NBT) photoreduction. The reaction solution (3 mL) contained 50 mM NBT, 1.3 mM riboflavin, 13 mM methionine, 75 µM ethylenediaminetetraacetic acid (EDTA), 50 mM phosphate buffer (pH 7.8), and 20 to 50 mL of enzyme extract. The reaction solution was irradiated under fluorescent light at 75 $\mu M.m^{-2}s^{-1}$ for 15 min. The absorbance at 560 nm was measured against a blank (non-irradiated reaction solution). One unit of SOD activity was defined as the amount of enzyme that inhibited 50% of NBT photoreduction.

CAT (EC 1.11.1.6) activity was measured following by measuring the decomposition of H_2O^2 as the decrease in absorbance at 240 nm. The reaction solution contained 50 mM phosphate buffer (pH 7.8) and 10 mM H^2O^2.

POD (EC 1.11.1.7) activity was measured following Chance and Maehly (Chance B., Maehly 1955) using 50 M pyrogallol, 50 mM H_2O_2, 1 mL of the 20 times diluted enzyme extract, and 5 mL of enzyme reaction solution containing phosphate buffer (pH 6.8). The assay mixture was incubated for 5 min at 25 °C, and the reaction was terminated by the addition of 0.5 mL of 5% (v/v) H_2SO_4. The spectrophotometer was used to measure purpurogallin production at 420 nm considering one unit of POD activity as the amount of purpurogallin formed per milligram of protein per minute.

The plant growth regulators, agar, and the chemicals used in this study were purchased from Sigma-Aldrich Co., St. Louis MO, and Duchefa Biochemie B.V., Haarlem, The Netherlands.

2.3. *In vitro* Rooting

Elongated and multiplied 2-3 cm long regenerated broad bean shoots were used for rooting. MS medium containing 1.0 mg L^{-1} Indole 3 acetic acid (IAA) was used for in vitro rooting. All cultures were kept at 25±2˚C in 16/8 h light/dark cycle in the growth chamber. Sixty (60) micro propagated shoots were used in each treatment divided into 12 replications containing 5 micropropagated shoots each (5 micropropagated shoots × 12 replications = 60 micropropagated shoots). Each experiment was replicated thrice. Data on frequencies of root induction, number of roots per plant, root length, number of shoot per explant and flower induction were recorded after three weeks of culture.

2.4. Ex vitro Acclimatization

The healthy plantlets of 6-8 cm length were taken out from the culture and washed in running tap water thoroughly so as to remove adhered agar-containing medium from the roots. Thereafter, plantlets were

transferred to potting mixture containing (a) clay loam soil, (b) peat moss, (c) perlite and (d) clay loam soil: peat moss (1:1) soil mix.

The clay loam soil used in the experiment had 42% (w/w) clay and 28% (w/w) sand with 49% water saturation percentage, CEC of 31 cmol kg^{-1}, EC 1.25 dS m^{-1}, 0.05% (w/w) total salts, pH of 7.8, 5.14% (w/w) lime, 138.4 kg ha^{-1} phosphorus, potassium of 1744.4 kg ha^{-1}, organic matter of 1.01% (w/w), total nitrogen of 0.09 % and organic carbon of 0.81%.

Peat moss used in the study was prepared locally from leaves. It had a pH of 6.2 and EC of 0.15 dS m^{-1}, the porosity of about (63% v/w), which allowed for high water absorption and had a low bulk density of 0.01 mg m^{-3}.

The perlite used in the experiment had a bulk density of about 53 kg m^{-3} and contained (w/w) 71 % SiO_2, 11% Al_2O_3 , 4% Na_2O, 2% K_2O, 0.5% Fe_2O_3, 0.2% MgO, 0.5% CaO and 4% loss on ignition (chemical/combined water).

The plants were transferred to 2-liter plastic pots containing 1.65-liter soil and peat moss mix and covered with transparent bags. Each pot was given 10 ml water after every two days. During the second week of culture when the plants began to show signs of growth; transparent bags were gradually removed and the plants were watered weekly depending on the conditions of plants. Greenhouse was maintained at 17±2ºC temperature and 69±2% relative humidity under 16 h light 8 h dark photoperiod.

2.5. Statistical Analysis

A total number of 60 explants were used for each treatment (regeneration and rooting) that were divided into equally distributed 12 replications. Data were subjected to one-way analysis of variance (ANOVA), and the post hoc tests were performed using Duncan's Multiple Range Test at 0.05 level of significance. The treatments were arranged in a completely randomized design.

3. Results

3.1. Antioxidant Enzyme Activities

Effects of hydropriming were evaluated by measuring SOD, CAT, and POD enzymatic activities on seedlings grown from non-water-soaked (control) and water-soaked seeds of cv. Eresen 87 and cv. Filiz 99 (Table 1). The results showed that SOD, CAT, and POD enzymatic activities of non-water-soaked seedlings (control) of both cultivars were higher compared to these activities on seedlings obtained after water soaking. Likewise, comparing two cultivars, these activities were significantly higher on cv. Filiz 99 irrespective of the treatment.

Table 1. Effects of the activity of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) on seedlings grown from non hydroprimed and hydroprimed seeds of faba bean cv. Eresen 87 and cv. Filiz 99

Treatments	Genotypes					
	SOD Activity (units·mg^{-1}·protein·min^{-1})		CAT Activity (units·mg $^{-1}$·protein·min $^{-1}$)		POD Activity (unit·min^{-1}·g^{-1}·FW)	
	Eresen 87	Filiz 99	Eresen 87	Filiz 99	Eresen 87	Filiz 99
Control	131.2^{a}	139.6^{a}	20.4^{a}	22.2^{a}	9.7^{a}	10.6^{a}
Hydropriming	113.5^{b}	127.0^{b}	19.1^{b}	17.1^{b}	7.4^{b}	7.9^{b}

Means followed by a different letters within a column for each parameter are significantly different at the 0.05 level of probability by *t* test.

3.2. Isolation of Explant

It was very difficult to obtain mature embryos from non-water soaked surface sterilized seeds, where it was very difficult to detach cohering seed cotyledons to approach mature embryos. Therefore, cohering cotyledons were cut opened closer to embryos using sharp blade without giving damage to them. These embryos were cultured on a 1 × MS medium containing 11 different concentrations of BAP + NAA for regeneration. The embryos did not survive except a few developing protrusions on the surface of explants due to the fast development of oxidative stress-related necrosis arrested growth and regeneration.

Contrarily, Water-soaked mature embryos on cv. Filiz 99 regenerated variable number of shoots on embryonic axis. Explants began to induce shoot initials after 6-7 days of culture. All cultures on cv. Filiz 99 induced shoots without callusing (Figure 1a); whereas, all explants of cv. Eresen 87 induced callus at embryonic axes followed by shoot regeneration (Figure 1b).

3.3. Shoot Regeneration

Regeneration from embryos taken from water soaked seeds ranged 80.0 to 100.0% on each of cv. Filiz 99 and Eresen 87. Shoot regeneration frequency varied on cv. Filiz 99; it remained 93.3 to 100.0% on all concentrations of BAP + NAA except one concentration (0.25 mg L^{-1} BAP + 1.75 mg L^{-1} NAA) with 80% shoot regeneration percentage (Figure 2). Shoot regeneration frequency on cv. Eresen 87 remained 100.0% on 6 concentrations of BAP+NAA concentrations, 93.3% shoot regeneration was noted on 2 concentrations of BAP+NAA concentrations and 80.0% regeneration was noted on 3 concentrations of BAP+NAA.

3.4. Number of Shoots per Explant

The number of shoots per explant ranged from 1 to 3.5 on cv. Filiz 99 (Figure 3).

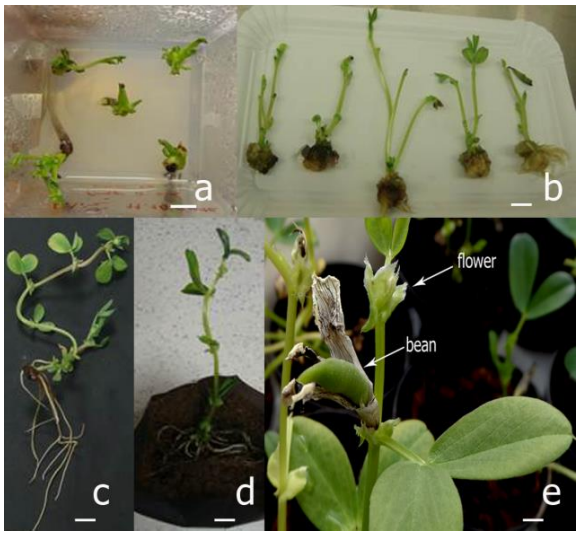

Figure 1. Shoot regeneration in broad bean from mature embryos obtained from water soaked (a) shoot regeneration on mature embryo explatsof cv. Eresen 87 (b) and cv. Filiz 99 (c) rooting of shoots obtained from cv. Ersen 87 (d) hardening of plants cv. Filiz 99 in plstic tubes (e) and their flowering and seed set.

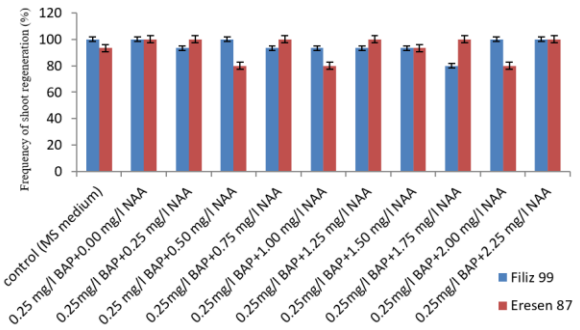

Figure 2. Comparison among average frequency of shoot regeneration percentage (%) of two broad bean varieties at different concentrations of NAA and BAP.

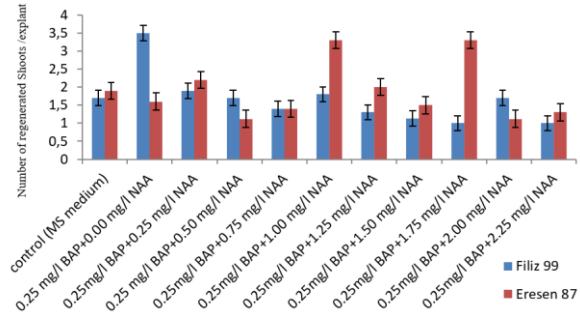

Figure 3. Comparison among average Number of regenerated shoots of two broad bean varieties at different concentrations of NAA and BAP.

The maximum number of shoots per explant was noted on MS medium containing 0.25 mg L^{-1} BAP. The rest of the cultures showed less than 1.9 shoots per explant. The number of shoots per explant showed inconsistency in two cultivars (Figure 3). The maximum number of shoots per explant on cv. Filiz 99 was recorded on MS medium containing 0.25 mg L^{-1} BAP. The maximum number of shoots on cv. Eresen 87 was noted on both MS medium containing 0.25 mg L^{-1} BAP + 1.00 mg L^{-1} NAA and 0.25 mg L^{-1} BAP + 1.75 mg L^{-1} NAA.

3. 5. Shoot Length

Shoot length per explant ranged 2.1 to 6.9 cm on cv. Filiz 99 and 1.7 – 6.5cm on cv. Eresen 87 (Figure 4). Maximum shoot length per explant for cv. Filiz 99 was 6.9 cm followed very closely by a shoot length of 6.4 cm on MS medium containing 0.25 mg L^{-1} BAP + 0.75 mg L^{-1} NAA and 0.25 mg L^{-1} BAP + 1.50 mg L^{-1} NAA (Figure 4). Minimum shoot length per explant for cv. Filiz 99 was noted on MS medium (control).

Shoot length on cv. Eresen 87 was inconsistent. Shoot length per explant ranged 1.7 – 6.5 cm on cv. Eresen 87. Maximum shoot length was noted on MS medium containing 0.25 mg L^{-1} BAP + 0.50 mg L^{-1} NAA. The rest of the concentrations of BAP+NAA never increased shoot length beyond 4.5 cm on any of the regeneration mediums. Minimum shoot length per explant was noted on MS medium containing 0.25 mg L^{-1} BAP + 0.25 mg L^{-1} NAA.

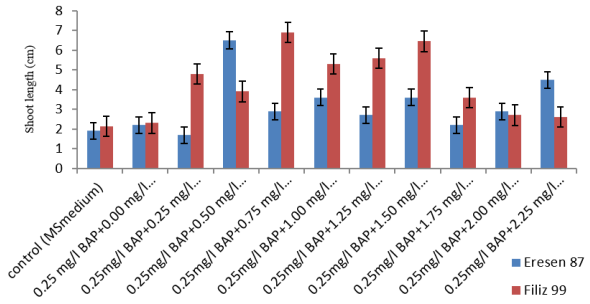

Figure 4. Comparison among average Length of regenerated shoots (cm) of two broad bean varieties at different concentrations of NAA and BAP.

3.6. Rooting and Acclimatization

The regenerating shoots were not difficult to root on MS medium containing 1 mg L^{-1} IAA. Profuse vigorous and strong root system with well-developed leaves was noted on all of the rooted shoots irrespective of the cultivar (Figure 1c). The plants had difficulty in acclimatization in clay loam soil, peat moss and perlite, and a mixture of perlite and clay loam.

A mixture of clay loam soil and peat moss not only helped in the development of a condition that was suitable for the growth and development of plants but induced a very suitable condition for the growth and development of plant roots (Figure 1d). There was no difficulty in the flowering of plants under shade house conditions. All acclimatized plants set seeds (Figure 1e).

4. Discussion

Broad bean is an important edible highly nutritious grain legume crop. It is prone to the number of biotic and abiotic stresses that affect its yield negatively. Broad

bean has the problem of self-incompatibility and has very little genetic diversity. Poor genetic diversity is a hindrance in the development of new varieties (Bond, 1987; Selva et al., 1989). The study aimed to identify suitable strategies for the regeneration of broad bean and its acclimatization under *in vitro* conditions.

Water soaking changes the internal environment of cells through osmosis by dilution with water. It is assumed that during imbibing of water and oxygen during hydropriming, the broad bean (Messina, 1999; Davila et al., 2003; Urbano et al., 2003; Vidal-Valverde et al., 2003) carbohydrates, phenolic compounds (phenylpropanoid acids, flavonoids, flavones, flavanones, and catechins Myricetin, Daidzein, Apigenin and Quercetin) and inhibitors like tyramine in the surrounding water (Zaats et al., 1987; Bekkara et al., 1998). It is accepted that this imbibition, dislocation, and elimination from seeds aid in the germination of seeds and seedling growth (Paul and Chodhury, 1991). It is also assumed that explants taken from these seedlings could enhance regeneration (Zaats et al., 1987; Bekkara et al., 1998; El-Mergawi et al., 2014; Yildirim, 2019). It is understood that the seeds undergo stress after secretion of phenolic compounds etc. that leads to the generation of reactive oxygen species (ROS: Superoxide anion radicals, hydroxyl radicals, H_2O_2, alkoxy radicals, and singlet oxygen) from non-water soaked seedlings. Consequently, these lead to lipid peroxidation-linked membrane deterioration (Jiang et al., 2001; Siddiqui et al., 2012). Whereas, water soaking-based management ended up with exudation of these compounds and lowering of oxidation activities as confirmed by SOD, CAT, and POD enzymatic activities and helped in overcoming oxidative damage in agreement with (Karray-Bouraoui et al., 2010; Mane et al., 2011; El-Lethy et al., 2013). This stress management in this study is supposed to help in the induction of rapid and large regeneration in this study avoiding plant cell death and transforming the saved energy to regeneration under *in vitro* conditions (Khalafalla and Hattori, 2000; Anwar, 2007; Anwar et al., 2010). It is assumed that broad bean seeds' germination and growth are in agreement with Bekkara et al. (1998). The results of the present study further suggest that phenolics could be avoided to a large extent if the seeds are water-soaked for 24 hours. They noted 100% exudation of these compounds from water-soaked seeds incubated at 30 °C. Whereas, in the case of non-water-soaked seeds, oxidative stress lead secretion of phenolic compounds and inhibitors resulted in deleterious effects on broad bean cell walls causing oxidative burst lead cell deaths.

This is in agreement with Böttinger et al. (2001), who has suggested that the difficulties of indirect regeneration from broad bean due to the accumulation of phenolic compounds, lead to cell death. They have further suggested that accumulation of the phenolic compounds could be avoided by frequent culturing explants on regeneration medium for 1 to 2 weeks. Therefore, broad bean regeneration and establishment from tissue culture are considered very difficult in general terms (Böttinger et al., 2001). It helped in the continuous division of cells and resulted in high regeneration percentage (80 -100%) in this experiment. Therefore, the results confirmed that if the quantity of oxidants in plantlets is diluted or reduced, there is no need to use antioxidants like ascorbic acid, glutathione citric acid etc. as has been reported by Abdelwahd et al. (2008) and Klenotičova et al. (2013), for reducing browning in broad bean.

In general except for a few cultures cv. Eresen 87 showed more genetic stability over cv. Filiz 99 in the frequency of shoot regeneration percentage.

This study describes a procedure to regenerate shoots from zygotic embryos using 2 broad bean cultivars without induction of callus. Callus induction is not desirable when true-to-type plants are desired. Direct shoot regeneration was noted on embryonic nodes on MS medium containing 0.25 mg L^{-1} BAP + different concentrations of NAA on two cultivars used in the study. All shoots developed directly from the embryonic nodes. The number of shoots per explant ranged from 1 to 3.5 on cv. Filiz 99 1.12 to 3.3 on cv. Eresen 87 respectively. This study has an edge over previous studies in terms of time to induce shoots, their rooting, and acclimatization that was achieved in 75 days. Previously a shoot regeneration system has been reported by Griga et al. (1987) through indirect somatic embryogenesis and organogenesis by Taha and Francis, (1990), Tegederet al. (1995) and Böttinger et al. (2001). Bahgat et al. (2009) used shoot tip and epicotyl explants and found possibility of development of embryos over a period of 14 months. They found that somatic embryos which were derived from shoot tips of cv. Giza 2 readily developed to fertile plants, while the somatic embryos developing on cultivar 24 Hyto were arrested at the torpedo stage and did not produce plantlets. The results are not in agreement with Bahgat et al. (2008). They regenerated somatic embryos on two Egyptian broad bean cultivars Giza 2 & Hyto using 10 mg L^{-1} BAP+0.5 mg L^{-1} NAA followed by transfer of the callus to 1/2× B5 medium. It is assumed that a high concentration of BAP used in the study may have caused stress on the explants that led to induction of somatic embryogenesis. High doses or prolonged exposure to phytohormones can cause damage to the vascular and other tissues (Kuplemez and Yildirim, 2020).

The results of this study report a good shoot length of 2.13 to 6.9 cm on cv. Filiz 99 and 1.7 – 6.5cm on cv. Eresen 87 has not been reported in previous studies (Griga et al., 1987; Taha and Francis, 1990; Tegeder et al., 1995; Böttinger et al., 2001; Bahgat et al., 2008).

Desjardins et al., (1987) described rooting of faba bean as a difficult procedure. Schulze et al. (1985), obtained 20% rooting only. This study reports 100% rooting under *in vitro* conditions. The broad bean plants regenerated under in vitro conditions on sucrose-containing media faced difficulties during acclimatization. They grew under high humidity, low light intensity, and limited gas exchange (Desjardins et al., 1987).

To identify strategies for successful acclimatization of these plants these were grown on different types of soil mix.

It was found that clay loam soil was unsuitable for acclimatization because of high percentage of clay in the substrate that hindered growth and development of roots. Moreover, the broad bean grows well in soils with pH of 7.0 -7.5. The soil used in this study may had high percentage of clay and lime, higher pH and poor organic matter that may have unsuitable effects on growth and development of tissue cultured plants that are very prone to external environmental conditions.

pH of peat moss used in the study was 6.2 that was unsuitable for growth and development of broad bean that require pH of 7.0 to 7.5 for growth and development. Perlite has ability to absorb and accumulate large amount of water. Perlite has ability to hold 200 to 600 percent of its weight in water. Broad bean is a plant that lives on moderate amount of water throughout its life cycle. Growing of tissue cultured plants in water saturated soils could lead to oxidation and cell death of roots. This may had caused earlier death in plants.

A mixture of perlite and clay loam may have resulted in development of a soil that was hard with large amount of water and plants died due to over moist soils related suffocation.

A mixture of clay loam soil and peat moss not only helped in development of a condition that was suitable for growth and development of plants but induced a pH and environment that was very suitable for growth and development of plant roots. This in conjunction with surrounding temperature and humidity helped the plants to grow flourish and acclimatize easily. Establishment of reliable acclimatization of tissue cultured plantlets (Goncalves et al., 1998; Gürel et al., 2019) permitted saving of time. This can also help by use of tissue culture for breeding, transformation and functional genomic studies.

5. Conclusion

Traits that are difficult to obtain with traditional breeding methods, such as insect resistance, may not be transferred from one plant species to another with traditional plant breeding methods. The results of this study suggest that water soaking improves broad bean seed germination and subsequently regeneration. The results could facilitate inbreeding using the single seed descent method and genetic transformation. In addition, tissue culture studies are used to obtain plants from gene-transferred cells.

Author Contributions

All task made by single author and the author reviewed and approved the manuscript.

Conflict of Interest

The author declared that there is no conflict of interest.

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